

# Sex differences in cooperative silk-spinning by weaver ant larvae

(ant caste systems/silk glands)

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**ABSTRACT** Final-instar larvae of weaver ants (*Oecophylla longinoda*) use their silk for nest construction rather than for cocoon spinning; this commitment represents a shift from entirely selfish to entirely cooperative behavior that occurred after the emergence of the phylogenetically advanced subfamily Formicinae. Male larvae were found to have smaller silk glands and to contribute substantially less silk to nest construction. The hypothesis of kin selection appears to be the most plausible and parsimonious explanation of this sex difference, but other, individual-level modes of selection have not been eliminated.

In the course of recent studies on the African weaver ant *Oecophylla longinoda* (1, 2), we noted a circumstance that may be of unusual interest in the consideration of the evolution of insect sociality. Throughout the formicoid ants, from the primitive subfamily Nothomyrmecinae through the great majority of phyletic lines in the advanced subfamily Formicinae, both female and male larvae use silk from their labial glands to spin protective cocoons just prior to pupation. This is essentially a selfish action shared with the nonsocial Hymenoptera, and males and females appear to be identical in the way they perform it. Only a few formicine species have curtailed or abandoned cocoon manufacture, including "weaver ants" of the genera *Dendromyrmex* and *Oecophylla* and a small percentage of the species of *Camponotus* and *Polyrhachis*. In these genera, the larvae contribute their silk to the construction of webs that bind and enclose arboreal nests. *O. longinoda* larvae have made a complete conversion: all of their silk is contributed to the colony's needs. Moreover, both the larvae and the adult workers that use them as shuttles display specialized behavior during the interaction: the larvae arch their bodies into a shallow S-shape and the workers lightly drum the heads of the larvae and the leaf surface in the near vicinity with their antennae.

A separate but possibly related and important circumstance is the fact that the male larvae, like the male larvae of all ant species, are less closely related to the other colony members than are female larvae. In the case of a singly inseminated mother queen, for example, the coefficient of relatedness among the all-female workers and between workers and virgin queens of the same generation is  $\frac{3}{4}$ , whereas it is  $\frac{1}{4}$  between queen-produced males and this generation of females and  $\frac{3}{4}$  between worker-produced males and the nonreproducing sisters of the egg-laying workers. Depending on the sex ratio of the colony, the average coefficient of relatedness of a male larva (averaged over all male larvae) to the remainder of the colony is therefore less than the average coefficient of relatedness of a female larva to the remainder of the colony. Even in the cases in which the queen has been multiply inseminated and produces all the male eggs (as our unpublished studies indicate is the case in *O. longinoda*), the males are less closely related to the remainder of the colony than are their female nestmates.

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A question of special significance thus arises in connection with the phenomenon of altruistic silk spinning: Have male weaver ants made less of a commitment to the colony's needs than their sisters? In the course of the evolution from cocoon spinning to nest building, a conflict between individual-lineage and kin selection seems inevitable. Every unit of protein converted into silk and contributed to nest construction is a unit withdrawn from personal growth. Where male and worker-destined larvae coexist and kin selection prevails, current theory (3, 4) predicts that the males will have more of an incentive to "cheat"—to hold back on the production of silk and allow the female larvae to carry a greater per capita share of the burden, because the latter individuals are subject to more intense kin selection. The question of interest can be restated as follows: Has kin selection been potent enough to forge a sexual difference in the evolution of larval cooperation during nest construction?

Prior to conducting any experiments, we noted the following three possible outcomes and their respective significance:

(i) If male larvae contribute less silk per unit weight than do female larvae, the hypothesis of prevailing kin selection would be supported.

(ii) If male and female larvae contribute the same amount of silk, the hypothesis would remain untested, because either kin selection did not occur or other selection pressures overwhelmed the effects of kin selection and brought about a sexual convergence in this trait—a pair of alternatives equally consistent with the data.

(iii) If male larvae contribute more silk than do female larvae, the hypothesis of prevailing kin selection would be contradicted.

## METHODS

Colonies and colony fragments of *O. longinoda* were collected in the Kilifi and Kwale Districts of Kenya and reared in moistened  $15 \times 2.2$  cm vials in the laboratory, according to procedures described elsewhere (5). Three queenright and three queenless units were used; in one case a queenright and queenless unit came from adjacent trees and were interpreted to be fragments of the same colony. Queenright colonies invariably gave rise to worker pupae, and queenless colonies (in which unmated workers laid the eggs) invariably gave rise to male pupae.

As a first step we measured the width of the head capsules and the total body length of 278 randomly selected larvae, ranging in developmental stage from those newly hatched from eggs to early prepupae. The frequency distribution of individuals based on the two measurements was apparently trimodal (Fig. 1), indicating that *O. longinoda* has at least three larval instars. Furthermore, both worker-destined and male larvae collected during the act of spinning proved to belong to the middle to late stages of growth within the terminal instar.

Silk glands of larvae were isolated in drops of insect Ringer's

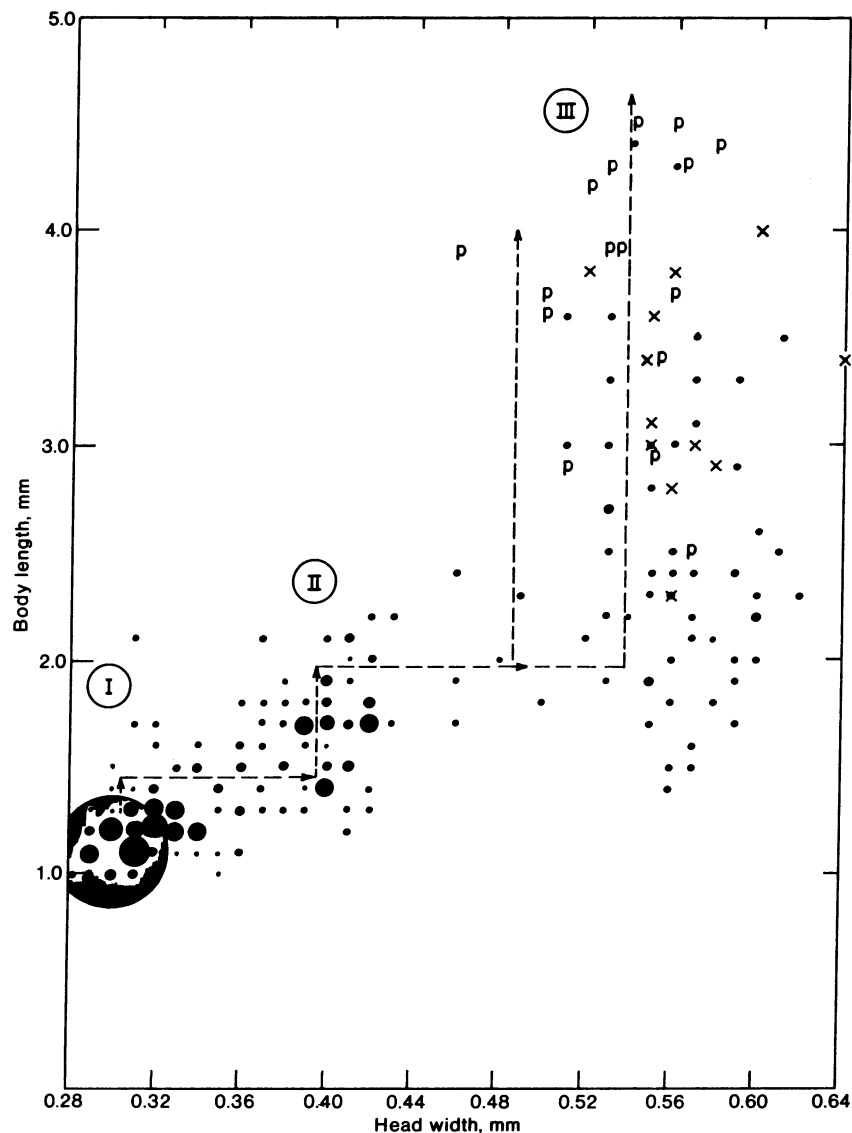


FIG. 1. Body proportions of 278 randomly chosen *O. longinoda* larvae, showing evidence of trimodality and the division of the larval stage into three instars. The overlap of size in spinning larvae (x) and larvae beginning adult development (prepupae, p) indicates that larvae contribute silk during the final instar. The diameter of the circles is proportional to the number of larvae at the body length and head width designated.

solution placed on a depression slide. They were found to be basically similar to those of at least some other formicine species (6), as follows. Each has a common duct that exits through a slit-like orifice in the center of the labium and divides internally into four branches. In most cases, two dorsal branches loop anteriorly for a short distance and then extend back along opposite sides of the dorsal surface of the midgut. A corresponding pair of ventral branches travels straight from the point of divergence to follow the ventral surface of the midgut. After the gland had been separated from the remainder of the body, it was stretched out lightly in the saline drops. The branches were then permitted to fall into natural positions, with the result that they were invariably twisted somewhat by the presence of relatively stiff bends and coils of the four branches. The "length" of the longest branch (usually one of the two dorsal branches) was taken as the length of the straight line running from the point of origin of the branch to its tip. The diameter of the widest part of the middle third of this same branch was also recorded. The two measures were then used to estimate the corresponding volume of a perfect cylinder, and this volume was defined as the "equivalent volume" of the longest branch

of the silk gland. Body length was measured as the greatest length along the long axis of the living larva after it had been allowed to relax into a natural posture while resting on its dorsum.

Performance of the larvae was measured in two ways. Vials containing various numbers of third-instar larvae were removed, labeled, cleaned of all silk, and replaced in their original positions. The number of workers using larvae as shuttles to replace the silk was then recorded at hourly intervals for periods not exceeding 4 hr during the first 12 hr after the original silk had been removed. Twenty-four hours later the total amount of new silk spun in the vial (Fig. 2) was scored as follows:

0, No trace of new silk.

1, A sheet covered half or less of the vial cross section; such constructions usually contained 500 threads or less.

2, A sheet covered more than half of the vial cross section, but it was still thin, containing 1000 or fewer threads.

3, Two thin sheets were present, containing together about 1000–5000 threads.

4, One heavy sheet covered half or more of the vial cross section and contained on the order of 10,000 or more threads.

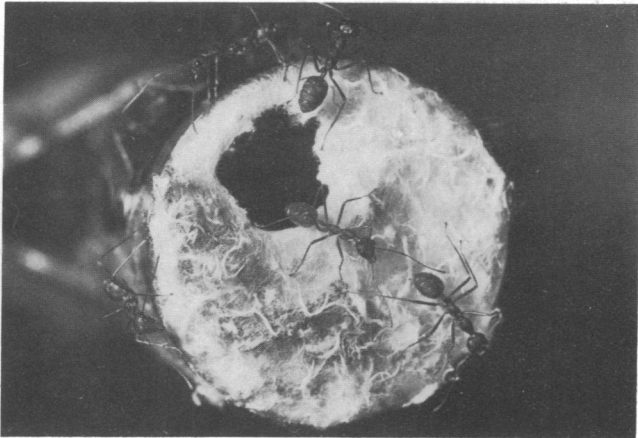


FIG. 2. Major workers of *O. longinoda* at the mouth of a nest vial, which has been mostly closed by a heavy layer of silk from worker-destined larvae.

5, Two heavy sheets were present, containing on the order of 10,000 or more threads.

In order to control for colony size, the colonies were chosen to vary in total size and in worker/larva ratio. And to determine whether the presence of the queen has an effect on size of the silk gland and spinning performance, we isolated an additional group of workers from a queenright colony, introduced worker-destined eggs for them to rear, and then repeated the procedures described above to obtain additional data on female larvae.

## RESULTS

**Volume of Silk Glands.** The measurements of the silk glands of final-instar larvae are displayed in Fig. 3 as a function of total body length, measured to the nearest 0.1 mm. For biological reasons the larvae can be divided into three classes: "pre-spinning" (body length  $\leq 2.5$  mm), "spinning" ( $2.6 \text{ mm} \leq \text{body length} \leq 3.5$  mm), and "prepupal" (body length  $\leq 3.6$  mm). The middle category contains most of the larvae of both sexes that are large enough to be used in spinning but still too small to display the degeneration of the silk gland that accompanies pupal development. The measured gland branches were next classified arbitrarily as small ( $\leq 0.02 \text{ mm}^3$ ) or large ( $> 0.02 \text{ mm}^3$ ) for each larva. We then arrayed the data for each larval size class into two-way contingency tables and tested the cells for independence. In the case of the spinning larvae, the behaviorally most important class, the sexual differences were highly significant ( $P < 0.0001$ ); in fact, the volume of the gland branches of the worker-destined larvae averaged 3 times that in the males. In the case of the pre-spinning larvae, the probability of independence was  $0.001 < P < 0.005$ ; in the case of larvae in the prepupal class, it was  $0.01 < P < 0.03$ . The smaller size of the male silk glands were confirmed by histological study of spinning-size larvae that were sectioned and then stained with azocarmine G. At the same time, we were unable to detect any additional differences at the tissue and cell levels.

**Participation in Silk Spinning.** Three colony fragments containing worker-destined larvae were compared with three fragments of comparable size containing male larvae. In 92 30-min checks of vials cleaned of silk but containing third-instar worker-destined larvae at the spinning stage, at least one larva was being used as a shuttle on 40 occasions and none on 52 occasions. In 147 30-min checks of vials cleaned of silk but con-

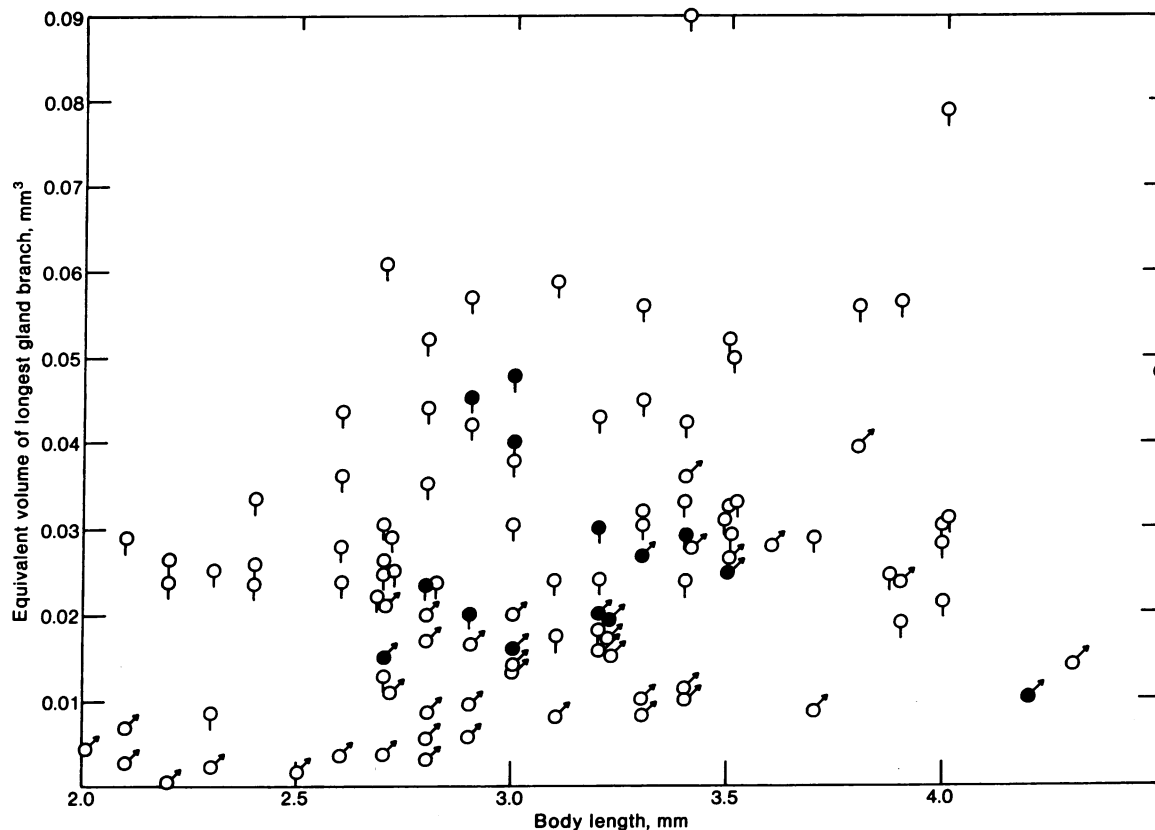


FIG. 3. Equivalent volume of the longest branch of the silk gland plotted against the length of the larva, which is also classified to sex. Not shown on this graph are the measurements of one large worker larva: body length, 4.3 mm, gland branch volume,  $0.12 \text{ mm}^3$ . ♀, Worker-destined; ♂, male. Solid symbol, collected while larva was spinning.

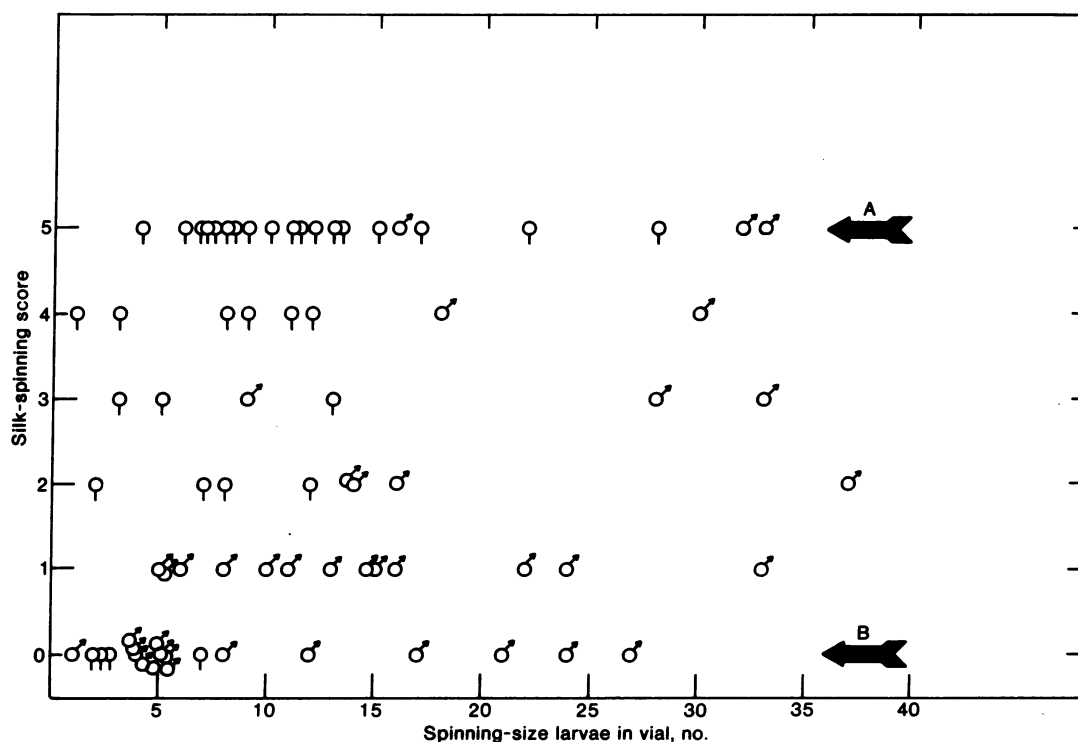


FIG. 4. Twenty-four-hour performances of worker-destined ( $\circ$ ) and male ( $\delta$ ) larvae plotted against the number of larvae in the groups observed. The scoring procedure is described in the text. Arrows: A, full double sheets finished; B, no silk spun.

taining comparable groups of spinning-sized male larvae, at least one larva was being used on 15 occasions and none on 132 occasions. The probability that this 4.3-fold greater activity of worker larvae was due to chance alone is less than 0.0001.

**Performance.** In Fig. 4 the scores achieved by groups of male and worker-destined larvae are plotted as a function of the number of third-instar larvae in each group. Here the difference between the sexes is most striking. When the data are arrayed in a two-way contingency table, with worker-destined and male larvae distinguished and performance partitioned into a low category (score: 0–2) and high category (score: 3–5), the probability that this strong a sexual difference could be due to chance alone is less than 0.0001. Moreover, a crude estimate of the average amounts of silk spun in the 24-hr period indicates that the worker larvae produced as much as 10 times the amount contributed by the male larvae.

**Worker-Destined Larvae Reared in the Absence of Queens.** Worker-destined larvae reared from eggs in the absence of a queen did not differ significantly in gland size or silk-spinning performance from worker-destined larvae reared with a queen. Thus, the sexual differences observed cannot be ascribed to the fact that the male larvae used in these experiments differed by virtue of being reared in queenless colonies.

## DISCUSSION

On the average, final-instar male larvae possess smaller silk glands, are used on fewer occasions by the workers, and, in the end, contribute far less silk than do final-instar female larvae. These differences in effect constitute the outcome of an evolutionary "experiment" that favors—but does not yet prove—the hypothesis of prevailing kin selection. Because of the relative phylogenetic lateness of the shift from cocoon spinning to nest construction in *Oecophylla*, many of the variables other than kin selection appear to us to have been controlled. For example, it can be argued that, prior to the or-

igin of higher social behavior in the aculeate behavior, adult male behavior and physiology had already specialized for reproduction to such an extent as to render males incapable of evolving fully cooperative and altruistic behavior. But this is not the case for larvae, which have undergone no earlier sexual divergence.

One explanation that nevertheless must be accommodated with the new evidence is parental manipulation (7). It can be argued that the queen "wishes" to have males that conserve protein selfishly and thus increase their reproductive potential, because they are a primarily reproductive caste. If manipulation occurs, it does not entail conflict because decreased silk production is already the expected best strategy from the point of view of the males. The evidence is consistent with this version of parental manipulation but the hypothesis is more complex than that of prevailing kin selection and requires an unknown control mechanism that must continue operating long after the death of the queen.

Other explanations of the sexual difference could be contrived, including pleiotropism, genetic drift, and unknown physiological requirements that are peculiar in adaptive goals to the development of *Oecophylla* males. We have considered in particular the possibility that the worker/male difference was not derived from sexually asymmetric kin selection but reflects instead some other distinction, as yet unknown, between workers and reproductive castes, the latter consisting of both the males and the queens. This causal relation appears unlikely to us for the following reason. Whereas adult reproductives are highly specialized in anatomy, physiology, and behavior for their essential non-labor roles, such is not the case for reproductive-destined larvae. Male larvae are nearly identical in outward appearance to worker larvae. Furthermore, although the possibility of fine tissue differences has not been discounted, routine dissections conducted at low magnifications together with limited histological comparisons have disclosed only one gross difference in internal anatomy—in the size of the silk

glands. Moreover, the silk glands are dismantled at the onset of adult development and hence do not serve as precursors to any of the organs that contribute to the adult reproductive roles. There is no known link between organ specialization at the larval stage and organ specialization at the adult stage. It is further true that in species belonging to formicine genera other than *Oecophylla* the pupal cocoons of males and workers are of approximately equal thickness and consistency. Thus, worker/reproductive divergence did not lead to a difference in silk production as a primary formicine trait independent of the social use of silk in nest construction. The 10-fold difference discovered in *Oecophylla* is evolutionarily derived and appears most efficiently explained by kin selection. We are keenly aware that the kin-selection hypothesis would be further strengthened if queen-destined larvae were also found to contribute more silk than male larvae. However, we have not yet been able to obtain queen-destined larvae to make the comparison and are not optimistic that such a comparison can usefully be made. Whereas third-instar male larvae are approximately the same size as third-instar worker larvae, queen-destined larvae at the same stage of development are many times heavier and are probably difficult for adult workers to manipulate as shuttles. For this reason, if queen-destined larvae were found to produce less silk the significance of the difference would be ambiguous.

Another potentially complicating feature of the experiment is the fact that the male larvae were all derived from workers in a queenless colony. There exists a possibility that as yet unknown physiological differences exist between such larvae and male larvae produced from queen-laid eggs or even any male larvae reared in the presence of the queen regardless of origin. While we know of no such difference that might affect the performance of the larvae in silk manufacture, the design of the experiment was necessarily such that we cannot rule it out on an *a priori* basis.

It is noteworthy that male weaver ant larvae have not given up the habit of silk spinning altogether. On the contrary, their silk glands are at least as proportionately large as those of the male and female larvae of species of *Formica* (ref. 6; unpublished data). Nor would they be expected to have gone this far. The silk they produce for the colony as a whole benefits themselves as well. Their selfish reward is greatest in queenless colonies, when all of the larvae are male and survival of the entire unit depends on male labor. Ten queenless fragments maintained in the laboratory produced either male brood ex-

clusively, which then contributed silk in the final larval instar, or no brood at all. This circumstance probably also occurs in nature, when colonies lose their queens or segments of queen-right colonies are isolated from their nestmates long enough to rear male broods. The important fact remains that male larvae produce much less silk than do worker larvae. In other formicine ants, silk production by the two sexes is apparently the same; that is, the cocoons of males and females just cover the pupa and appear to be of the same thickness and quality. But in *O. longinoda*, in which all of the silk is committed to social use, there is at least a 10-fold difference in the quantity produced.

The apparent evolutionary compromise reached by the *Oecophylla* male larvae has an interesting parallel in the behavior of adult males of the carpenter ants *Camponotus herculeanus* and *C. ligniperda*. These insects are unusual in that they remain in the nest for 9–10 months, conducting nuptial flights early in the season after overwintering, whereas the males of most other ant species leave the nest soon after eclosion. The *Camponotus* males are also unusual in that they receive food from other adult colony members and regurgitate it freely (8). Like the spinning male larvae of *Oecophylla*, they have evolved a limited mode of cooperative behavior under special circumstances.

It will be of considerable theoretical interest to learn whether similar male–female dimorphism occurs in other forms of larval contribution to colony welfare, in particular the donation of larval salivary secretions to adult workers, males, and queens in the wasps and ants (9).

We are grateful to several readers for helping us evaluate alternative explanations of the behavioral dimorphism but especially to Mr. Alex Mintzer who effectively emphasized the possible existence of a more fundamental worker/reproductive (as opposed to worker/male) difference. This research has been supported by Grants BNS73-00889 and BNS77-03884 from the National Science Foundation.

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